Herceptin®
(Trastuzumab)

Oncologic Drugs
Advisory Committee Meeting

December 5, 2001

Genentech, Incorporated

Introduction

Marianne Armstrong, Ph.D.

Sr. Director, Regulatory Affairs
Genentech, Incorporated

Purpose

To seek approval of Genentech's sBLA that **requests inclusion of fluorescence *in situ* hybridization (FISH) testing using the PathVysion™ HER2 DNA Probe Kit** (Vysis, Inc.) **in the current Herceptin label** as a diagnostic method to select patients for Herceptin therapy

Herceptin Profile

- Herceptin is a recombinant DNA-derived humanized monoclonal antibody that targets HER2, the protein product of *c-erbB-2*.
- More than 60,000 women worldwide have received Herceptin since market introduction.

Regulatory History

- Herceptin was approved in September 1998 for:
  - First line treatment in combination with paclitaxel in MBC patients whose tumors overexpress HER2.
  - Second- or third-line, single agent therapy in MBC patients whose tumors overexpress HER2.

Regulatory History

- The only FDA-approved diagnostic method to aid in the selection of patients for Herceptin therapy is immunohistochemistry (IHC).
- The two FDA-approved HER2 IHC diagnostic kits include the HercepTest® (DAKO, Inc.) and Pathway™ (Ventana, Inc.).
- Only the HercepTest is included in the Herceptin package insert.

Presentation Overview

Today we will:

- Present data that demonstrate PathVysion, a HER2 FISH kit, is an appropriate method to aid in the selection of patients for Herceptin therapy.

This data will include:

- HER2 biology and the scientific rationale
- Concordance data from the Herceptin clinical trials database
- Exploratory clinical outcomes analysis from the Herceptin clinical trials database

Agenda

Marianne Armstrong, Ph.D.
Sr. Director, Regulatory Affairs
Genentech, Inc.

Introduction

Michael Press, M.D., Ph.D
Professor, Dept. of Pathology
Harold E. Lee Chair for Cancer Research
Norris Comprehensive Cancer Center
University of Southern California

HER2 Biology and Methods of Assessment

Robert Mass, M.D
Assoc. Director, Medical Affairs
Genentech, Inc.

Concordance & Clinical Outcome Analyses

Conclusions

Summary

Our goal today is to demonstrate that PathVysion is an appropriate method to aid in the selection of patients for Herceptin therapy.

HER2 Biology and Methods of Assessment

Michael Press, M.D., Ph.D.

Professor
Harold E. Lee Chair for Cancer Research
Department of Pathology
Norris Comprehensive Cancer Center
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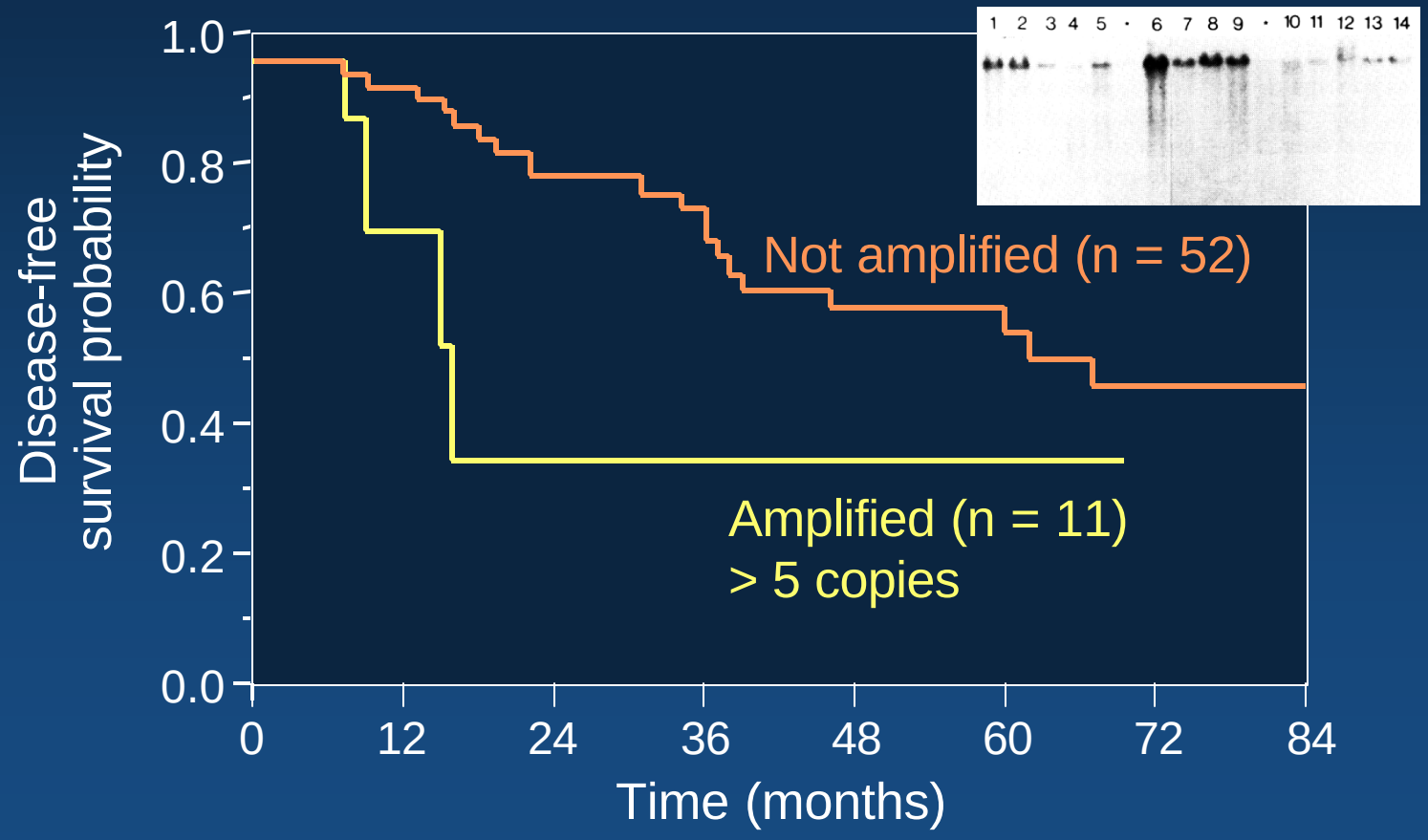
HER2 Biology and Methods of Assessment

- HER2 biology
- Immunohistochemistry (IHC)
- Fluorescence *In Situ* Hybridization (FISH)
- Clinical significance

Clinical Implications of HER2/*neu* Amplification

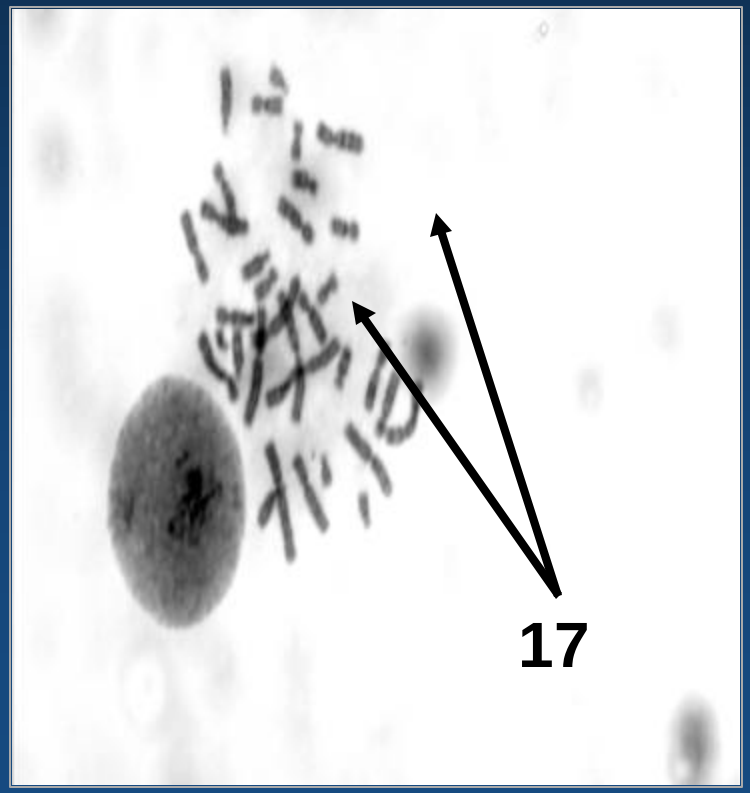
Node-positive patients with no amplification vs
node-positive patients with greater than 5 copies of HER2/*neu*



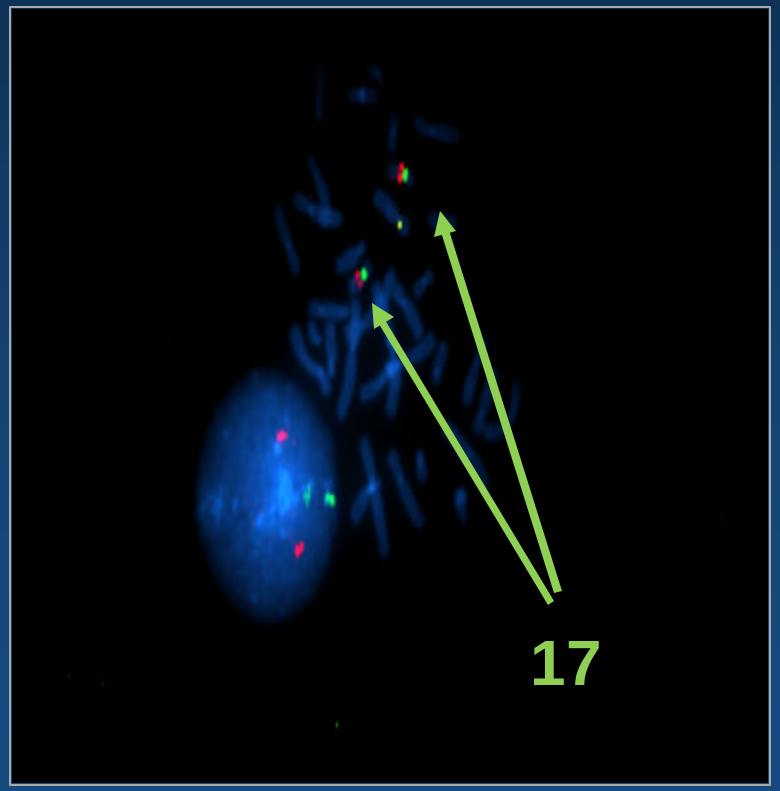
Slamon et al., Science 235:177-182, 1987

Localization of HER2/*neu* Gene on Chromosome 17

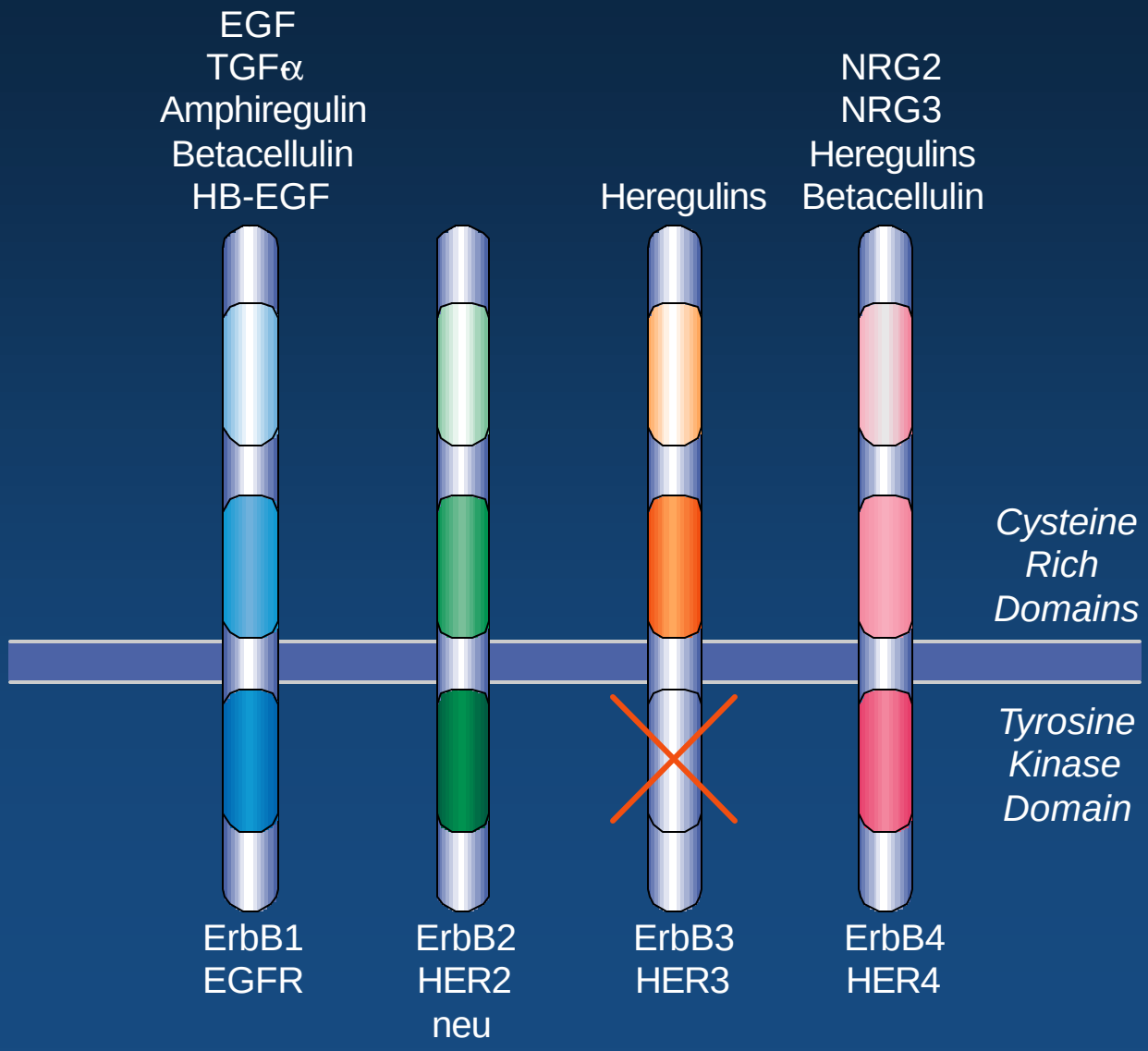
Normal interphase nucleus and metaphase spread



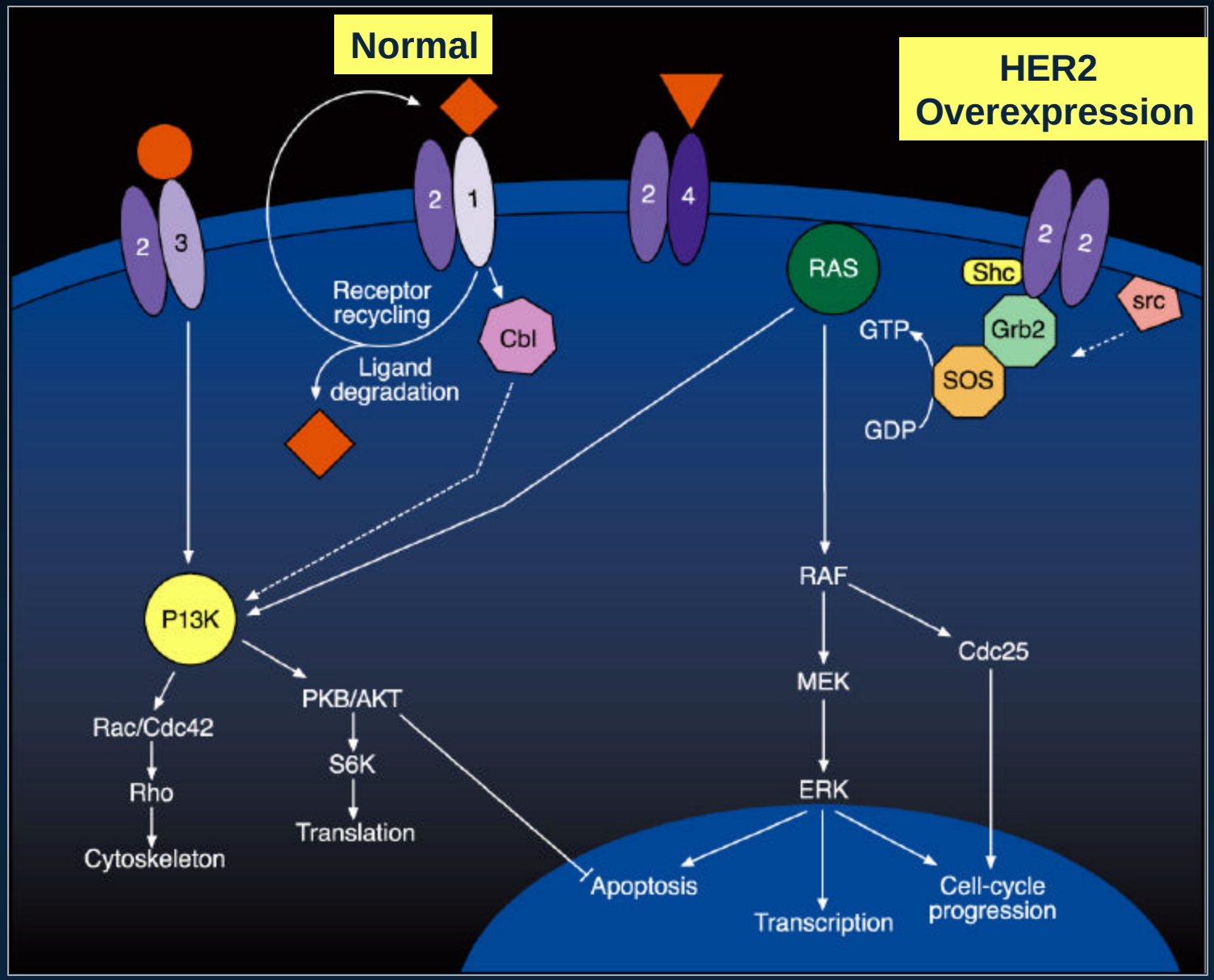
HER-2/*neu*
Chromosome 17 centromere



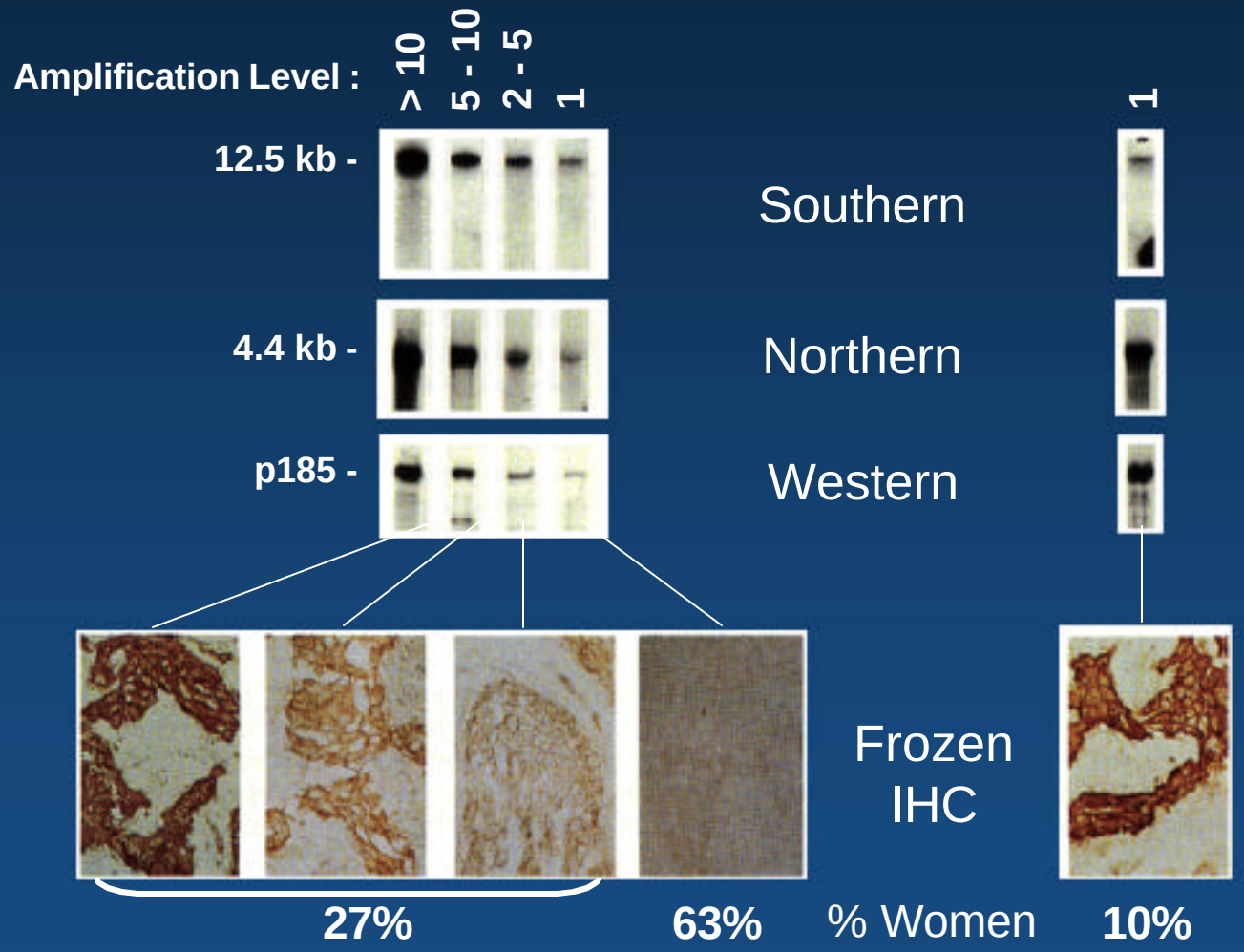
Epidermal Growth Factor Receptor Family



HER2 Biology

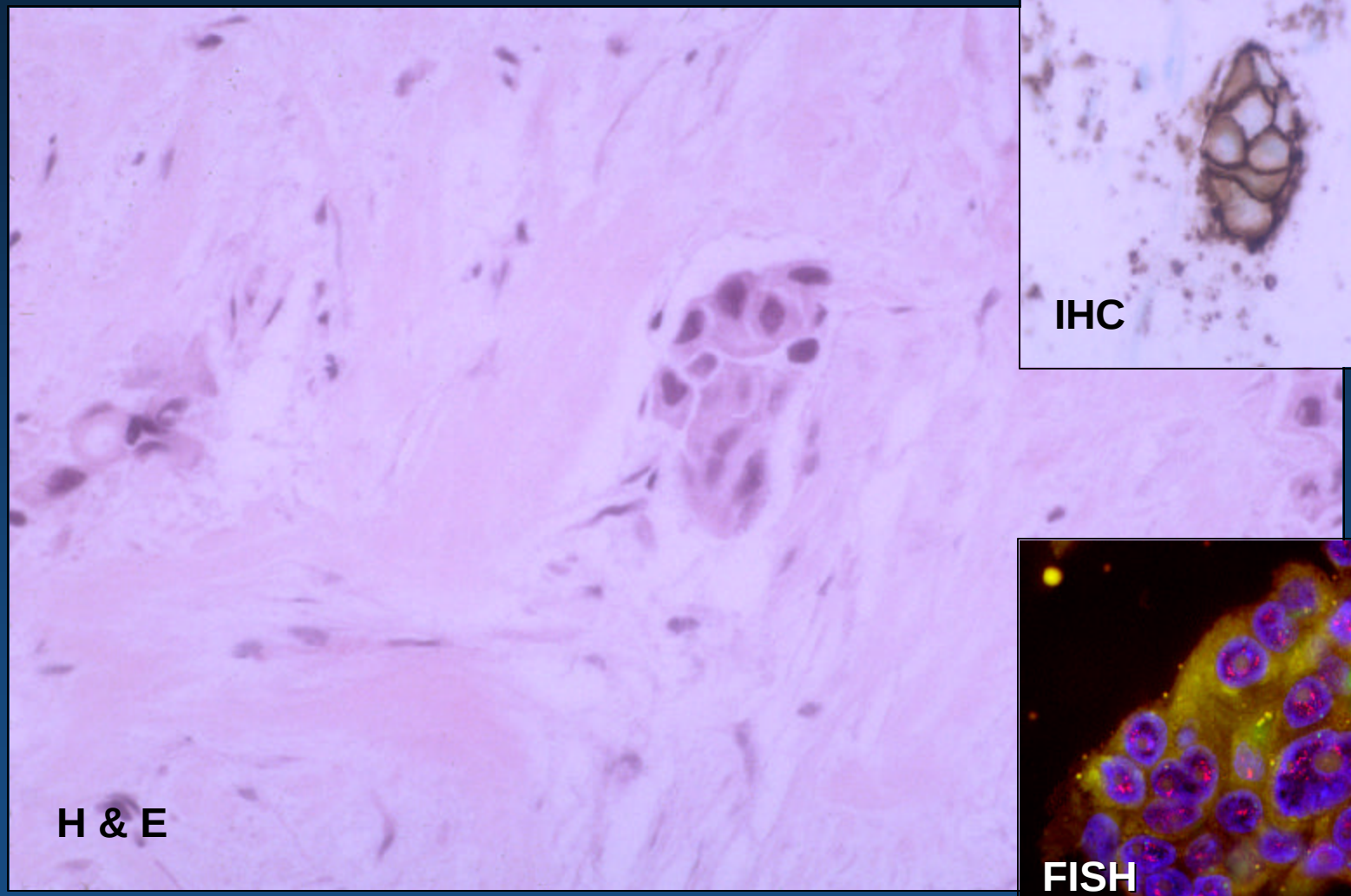


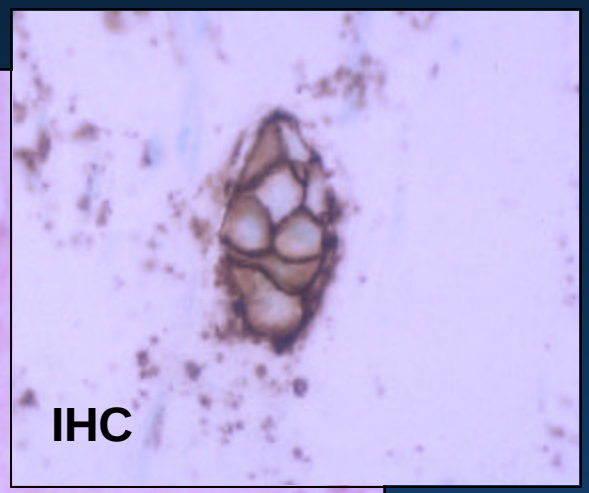
Correlation of HER2/*neu* Gene Amplification with Overexpression

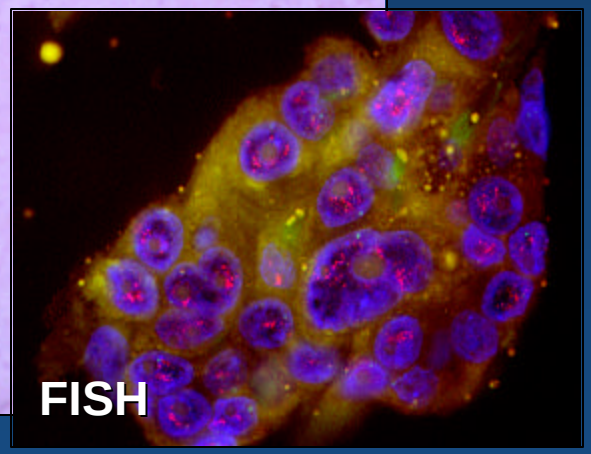


Slamon et al., Science 244: 707-712, 1989

Single Copy Overexpression

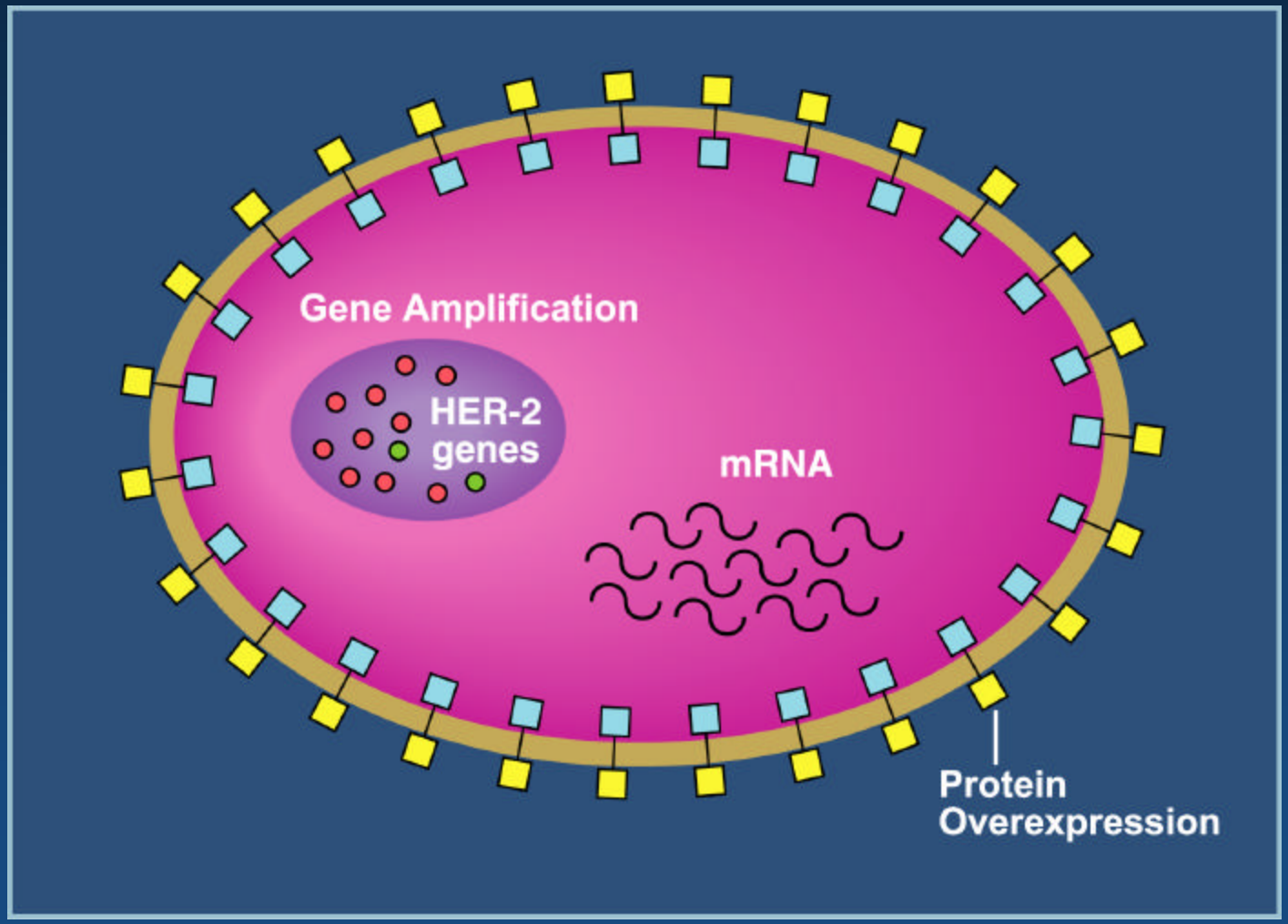






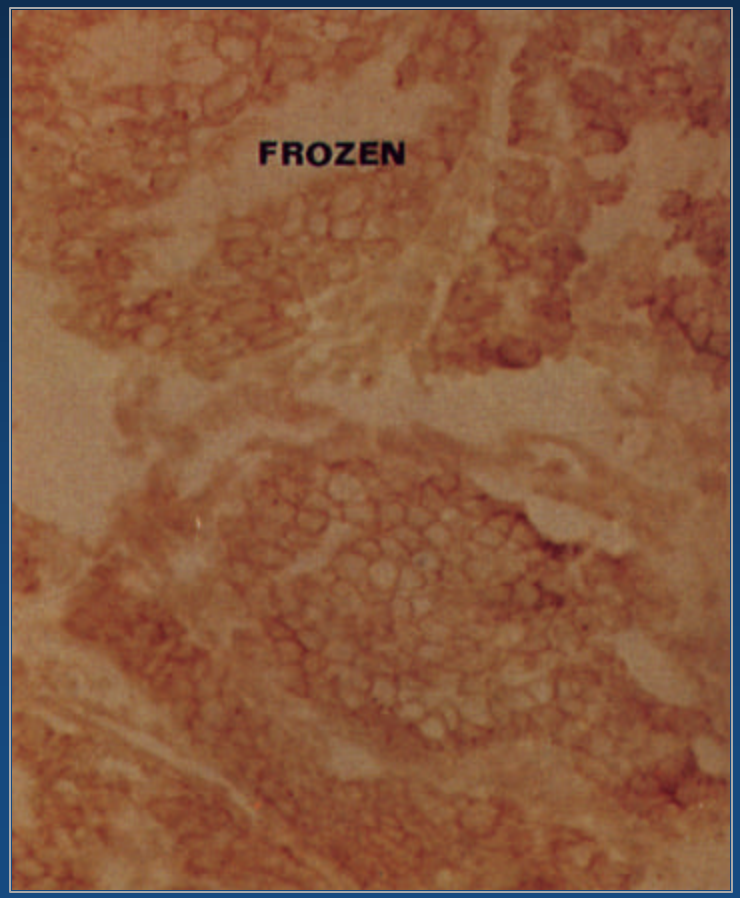
Slamon et al., Science 244:707-712, 1989; Pauletti et al., Oncogene 13:63-72, 1996

HER-2/*neu* Gene Amplification is Responsible for Overexpression

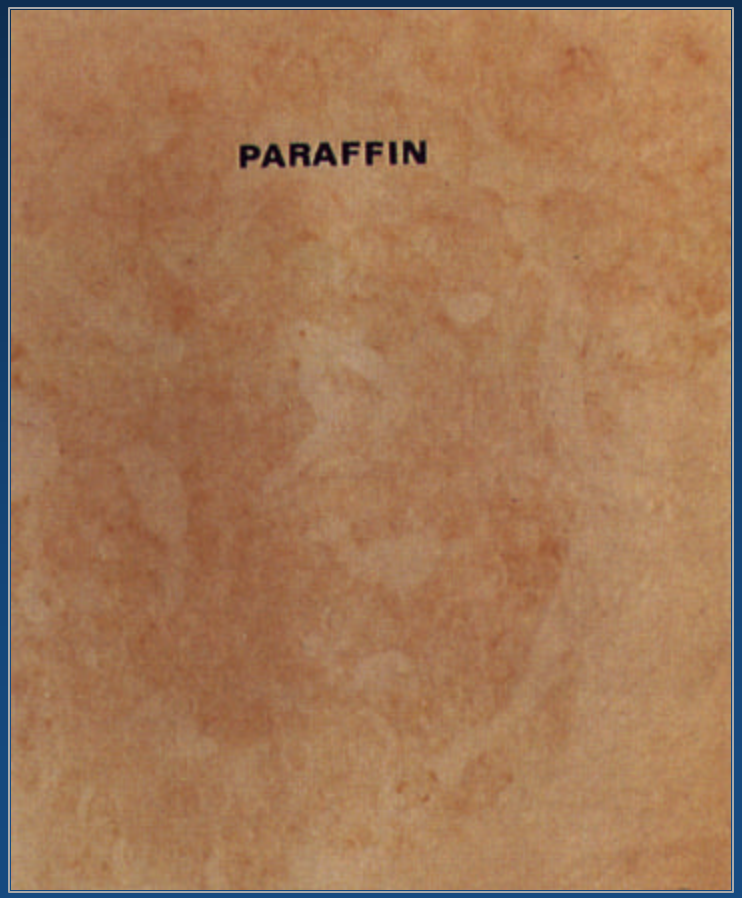


Fixation and Paraffin Embedding Result in Decreased Antigenicity

2 to 5 fold Amplified Frozen IHC



2 to 5 fold Amplified/Fixed, Paraffin IHC

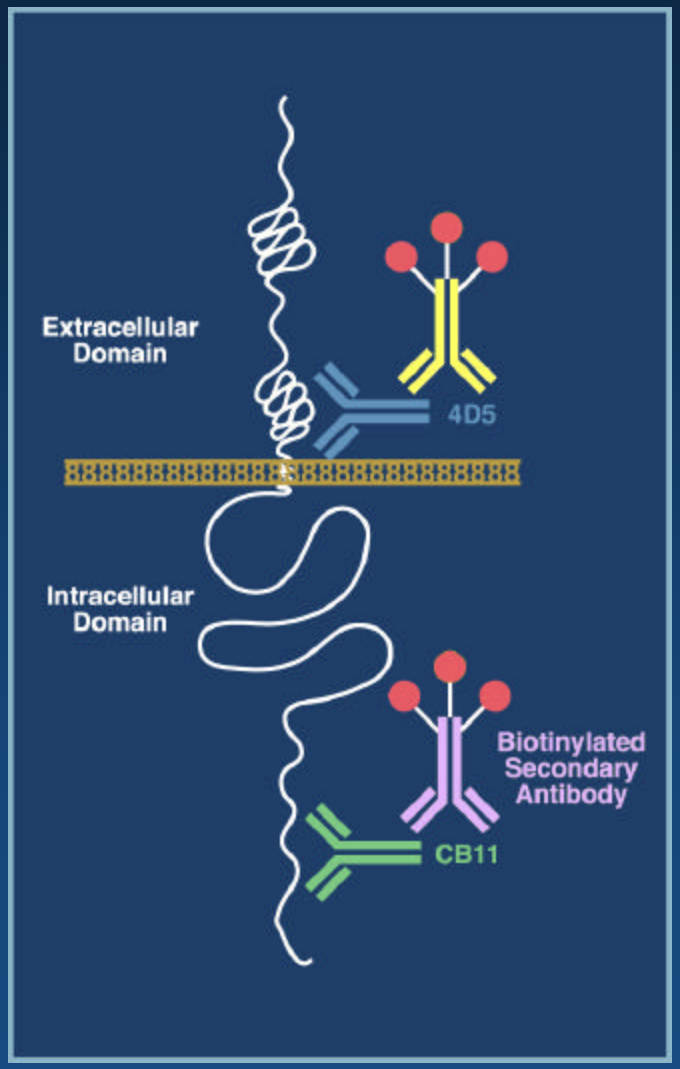


Slamon et al., Science 244:707-712, 1989

Immunohistochemistry: Clinical Trial Assay

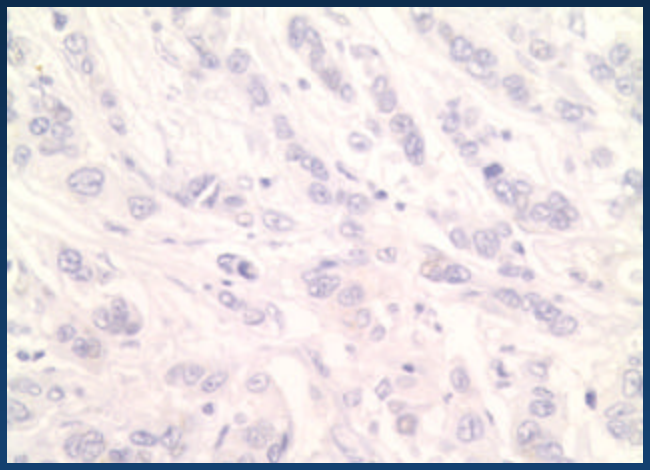
Key Features:

- Primary antibody - two different monoclonals
  - 4D5 and CB11
- Procedure - indirect avidin-biotin for each antibody
- Antigen retrieval
  - 4D5 - protease digestion
  - CB11 - microwave

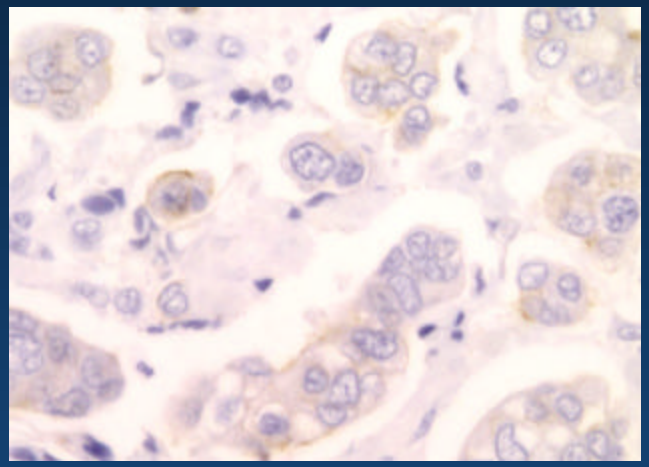


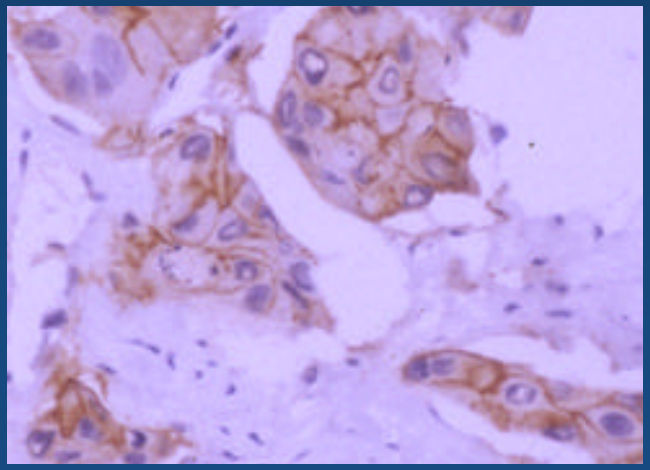
HER2 Overexpression Detection by Immunohistochemistry

Negative or, 0+

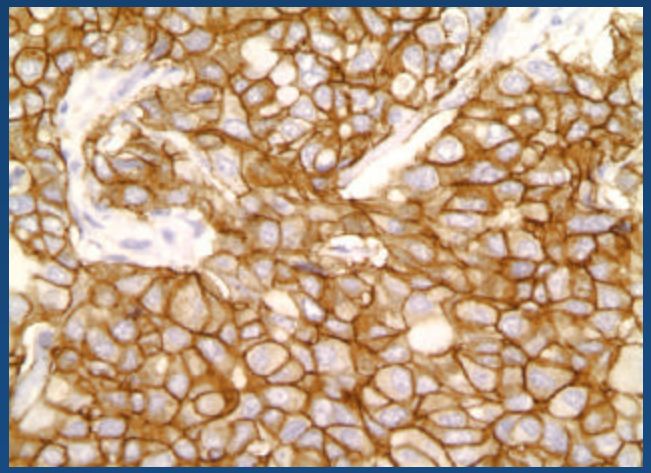


1+





2+



3+

Detection of HER2 Protein by Immunohistochemistry

Pros

- Widely available
- Rapid procedure
- Light microscope based
- HercepTest™ and Pathway™ FDA-approved assays for Herceptin eligibility selection

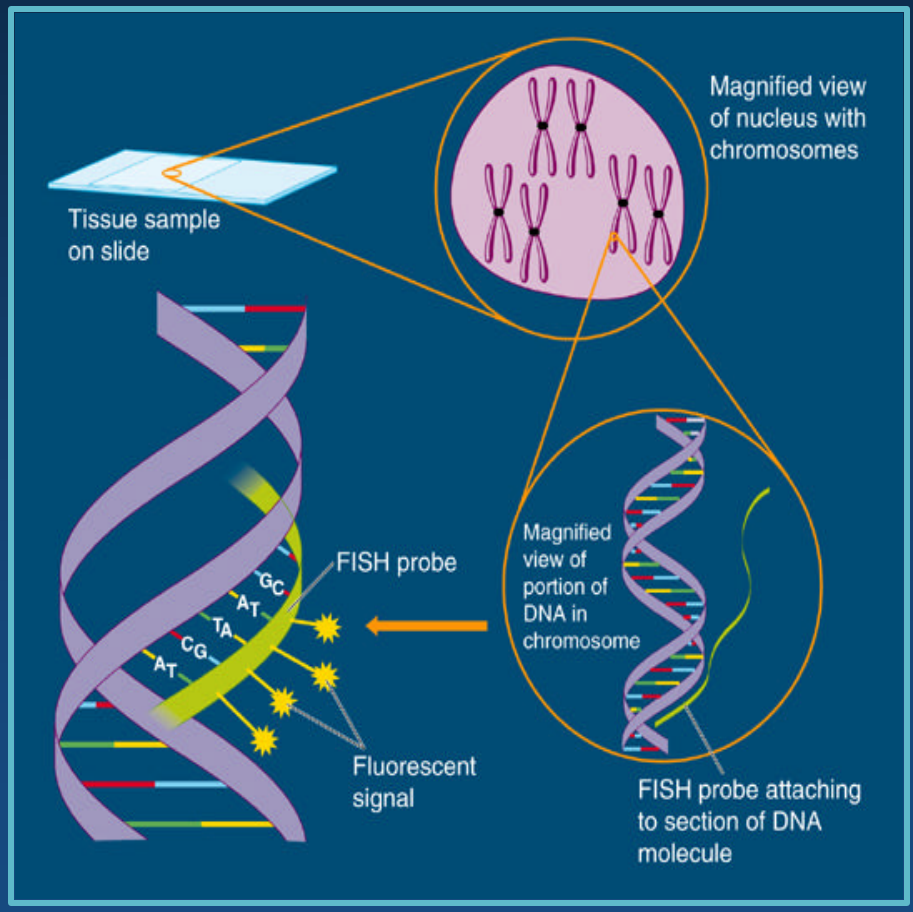
Cons

- Variable antibody sensitivity and specificity
  - Highly impacted by tissue processing variables
  - Affected by antigen retrieval and reagent variability
- Non-FDA-approved assays in routine use
- Subjective scoring criteria
  - Low pathologist concordance and high interlaboratory variability

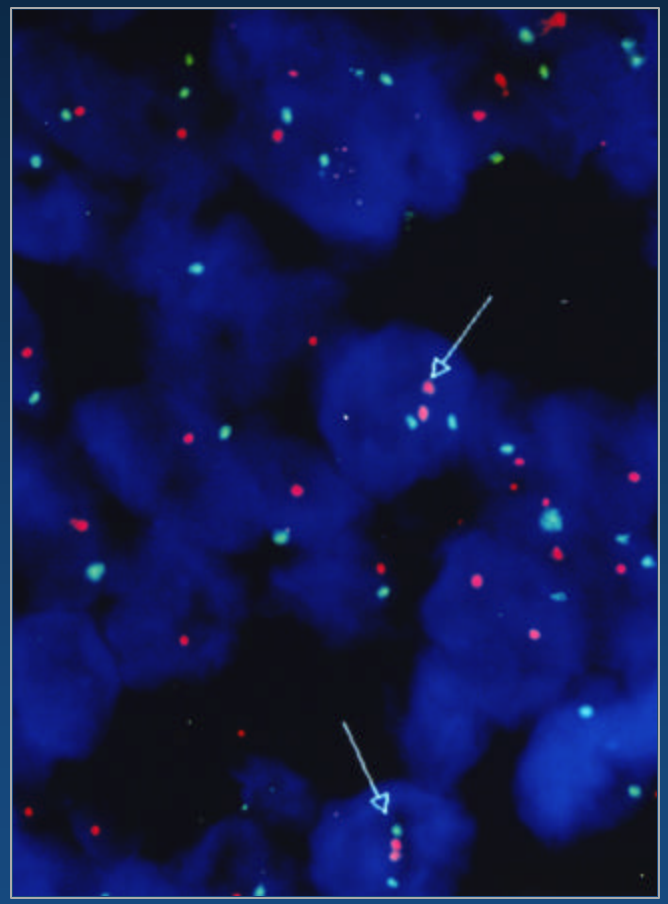
Fluorescence *in situ* Hybridization: PathVysion

Key Features:

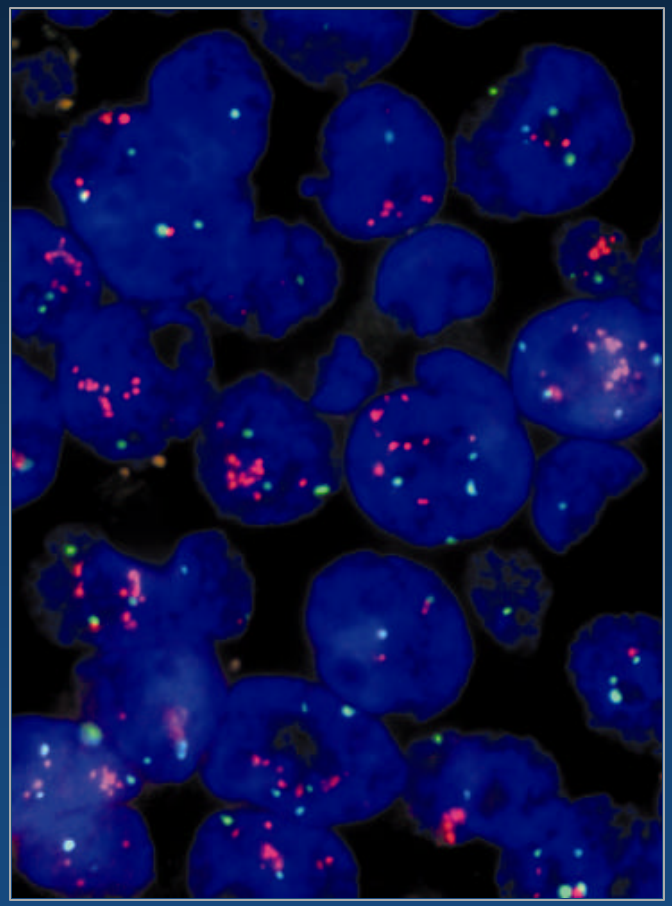
- Probes
  - Direct labeled
  - HER2 sequence
  - Chromosome 17 centromere
- Interpretation
  - Signal enumeration
  - Ratio of HER2:Chr 17 signals



HER2/*neu* Gene Assesment by FISH

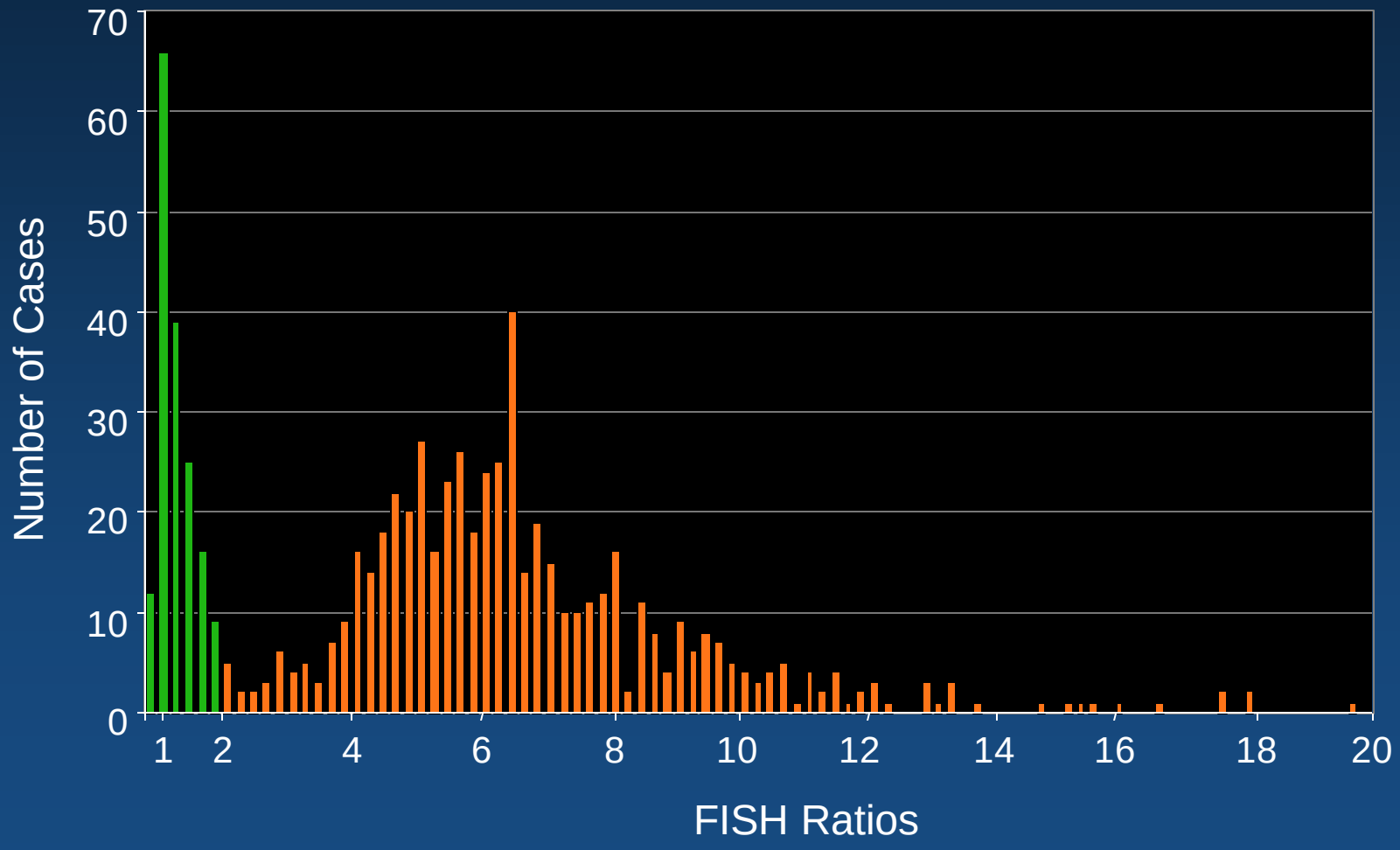


< 2.0 Not Amplified
(FISH-)



≥ 2.0 Amplified
(FISH+)

Population Distribution of FISH Scores



Use of FISH to Measure HER2 Gene Copy Number

Pros

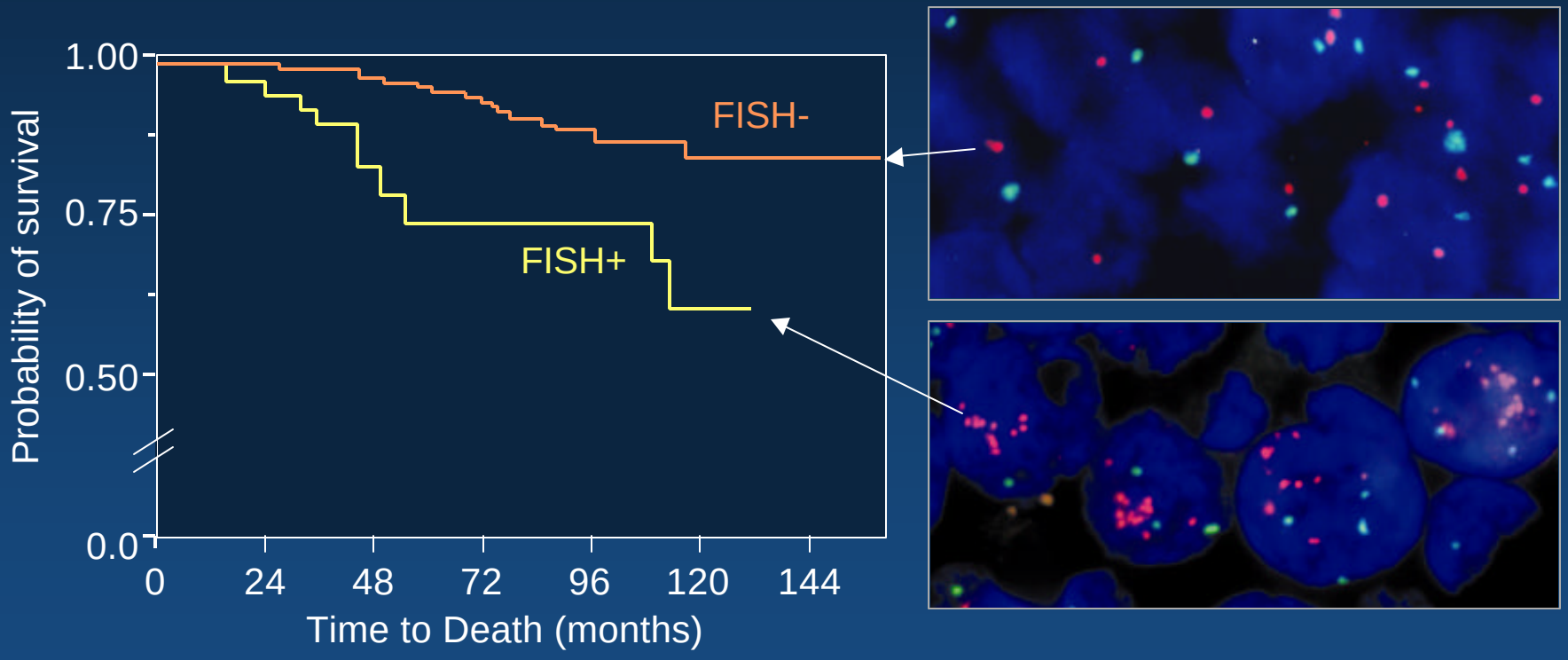
- DNA is a stable target
- Standardized threshold for positivity
- Built-in internal control
- Low interlaboratory variability
- High accuracy (sensitivity and specificity)

Cons

- Fluorescence microscope equipped with correct filter sets is required
- Certain fixatives interfere with assay (non-informative result)
- Limited community experience with tissue-based FISH

Clinical Significance

HER2/*neu* Gene Amplification Associated with Poor Outcome in Node-negative Breast Cancer

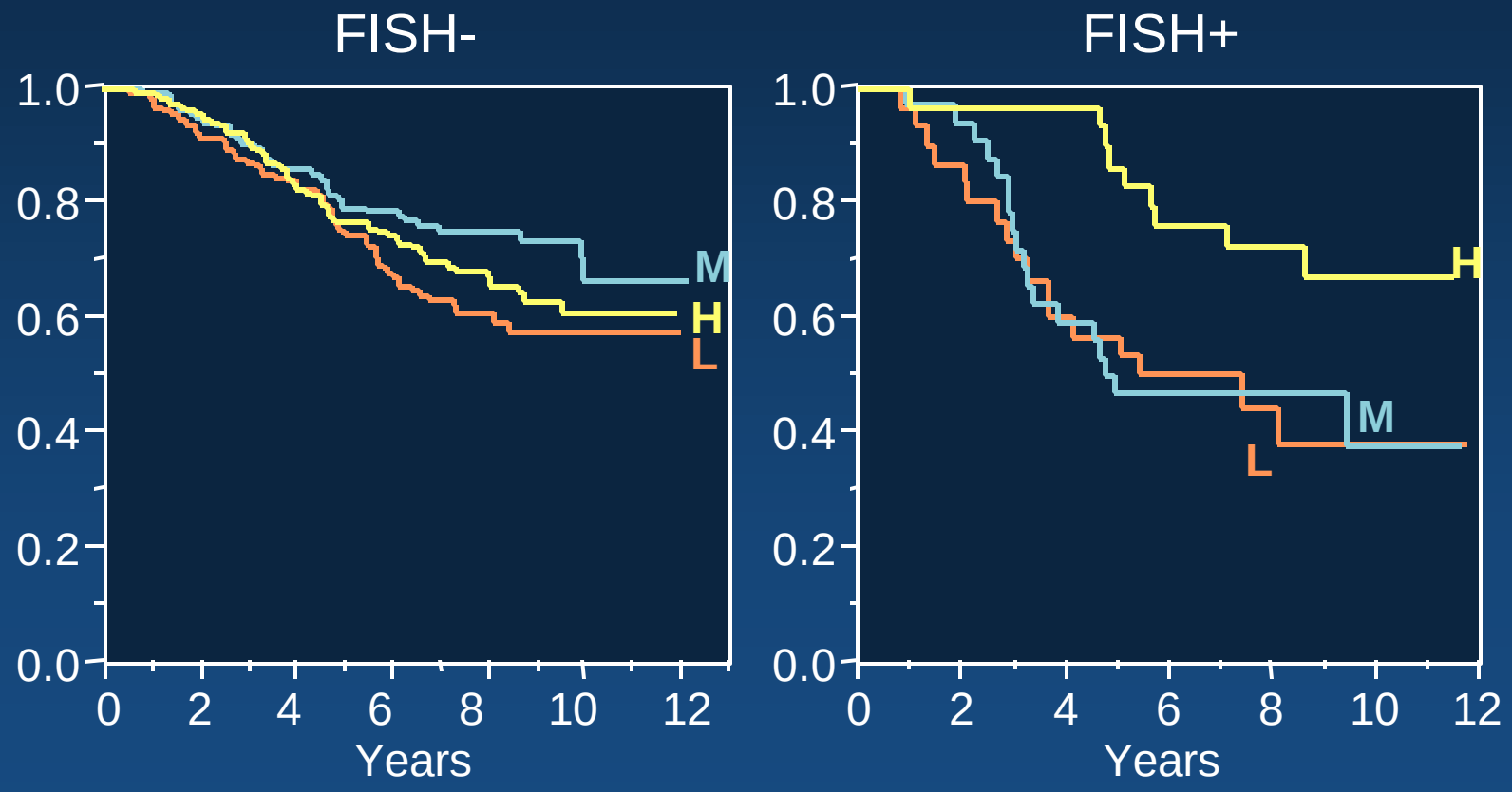


Press et al., J. Clin. Oncol. 15:2894-2904, 1997

Clinical Significance

HER-2/*neu* Gene Amplification by FISH is Predictive of Response to "High-Dose" Adriamycin Chemotherapy

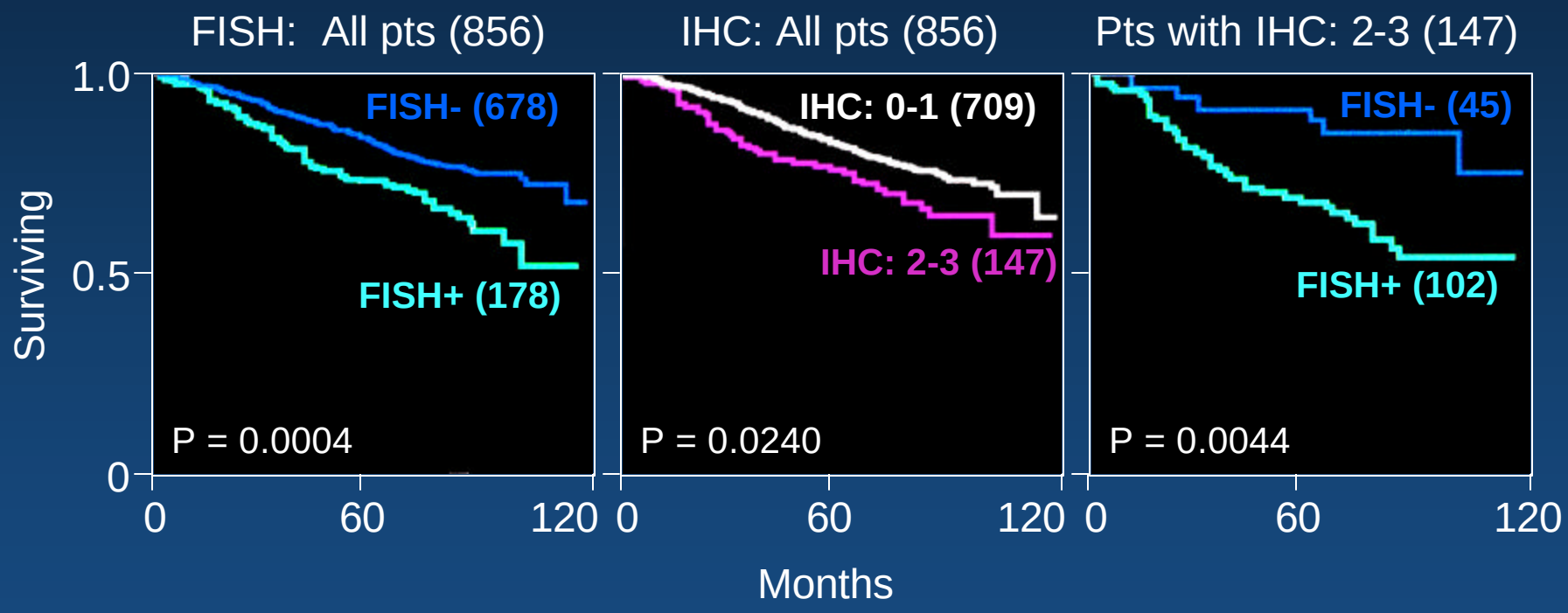
Overall survival



Muss et al., NEJM, 330:1260, 1994 and Vysis PathVysion PMA, 1998

Clinical Significance

Comparison of Overall Survival in FISH+ / IHC+ versus FISH- / IHC+ Breast Cancer



Pauletti et al, J Clin Onc, 21:3651-3664, 2000

Conclusions

- Direct correlation exists between gene amplification and overexpression
- FISH is a robust method for detecting gene amplification
- Amplification, as determined by FISH, is a clinically meaningful measure associated with poor prognosis and predictive of therapeutic response

Robert D. Mass, MD

Associate Director, Oncology

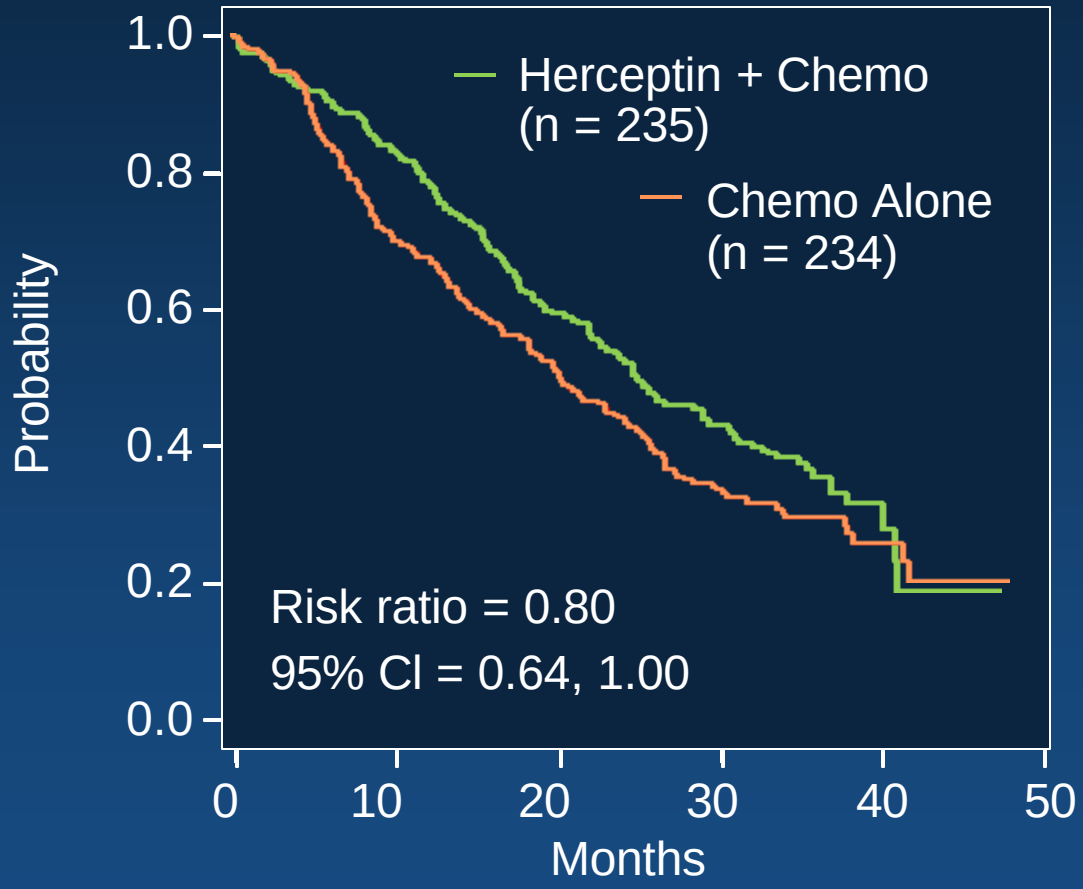
Genentech, Inc.

Rationale

- Fundamental biologic link between HER2 amplification and protein overexpression
- PathVysion has the ability to provide both prognostic and predictive information in human breast cancer
- IHC, the only FDA approved methodology to select patients for Herceptin therapy, appears to have significant accuracy issues when applied to formalin fixed clinical material

Survival

Chemotherapy +/- Herceptin, 1st line MBC



PathVysion will provide an alternative, non-IHC assay method to accurately identify patients for Herceptin therapy

Introduction

Goal: to provide data supporting the addition of PathVysion (FISH) to the Herceptin label to identify patients for Herceptin therapy

Two studies support the label supplement

- *Concordance Study*
  - Concordance between PathVysion and the Herceptin Clinical Trials Assay (CTA)
- *Clinical Outcomes Study*
  - Clinical outcomes analysis assessing FISH status in the pivotal Herceptin trials
  - Interlaboratory validation assessment

Source of Tissue Specimens

- The Herceptin pivotal trials represent the only large database available to correlate HER2 diagnostics with treatment outcome
- Both studies utilized archived tissue sections that had been stored for 2 to 5 years

Objective: To establish the concordance between the CTA and FISH (PathVysion)

Methodology

- **Prospectively defined study utilizing clinical trials samples that were retrospectively tested with PathVysion**
- **Single blinded**
  - **Analysis plan identical to the HercepTest concordance protocol used for FDA approval**
    - **1:1 positive:negative sample ratio**

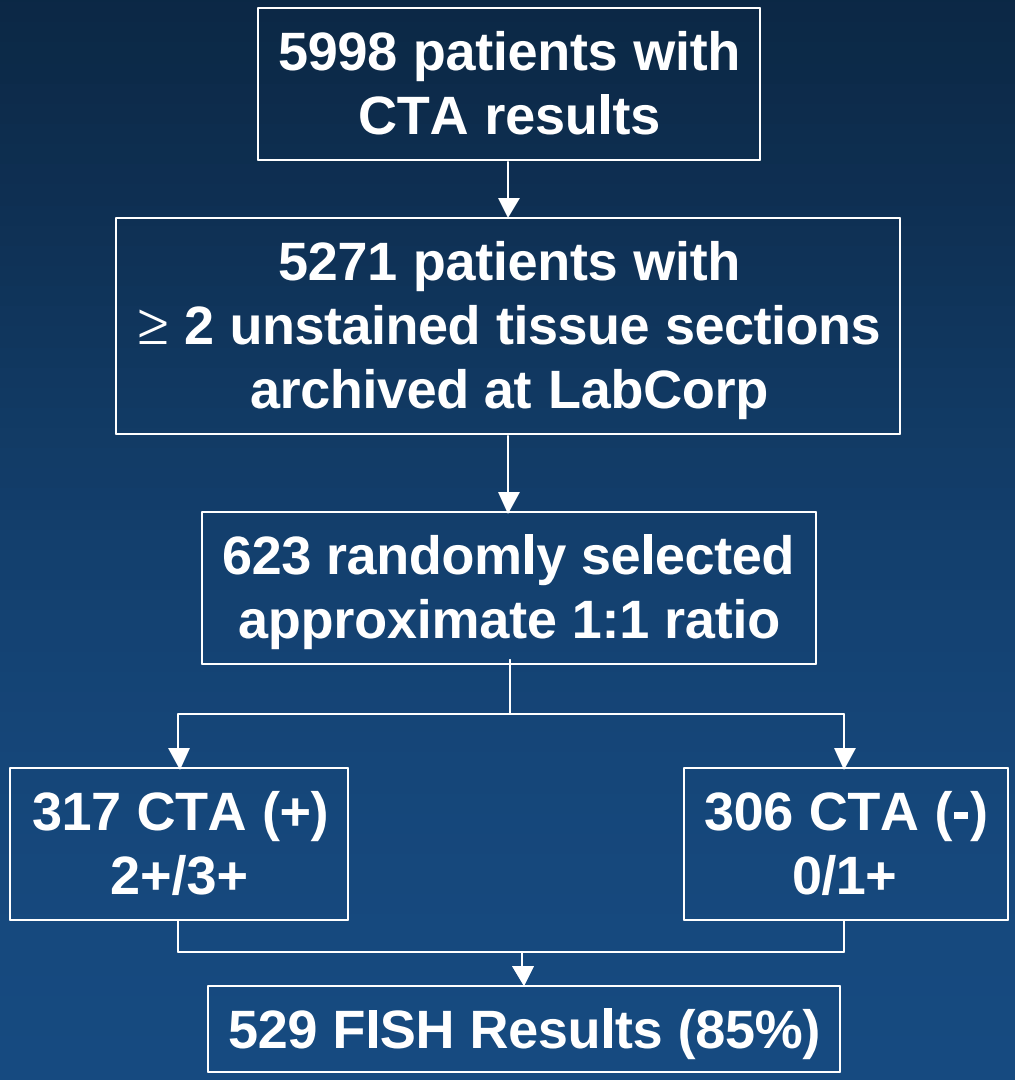
      positive (CTA 2+/3+)

      negative (CTA 0/1+)
    - **Provides maximal statistical power to assess concordance**
- **FISH positive defined as HER2:CEP17 ratio ³ 2**

Statistics

- **Primary endpoint**
  - **Concordance in 1:1 population**
- **Secondary endpoints**
  - **Concordance extrapolated to the clinical trials population**
  - **Kappa statistic**
- **Assumptions**
  - **Concordance £ 75% was pre-specified as ‘unacceptable’**
  - **90% power to detect 5% improvement over that ‘unacceptable’ level (£ 75%)**
  - **1-sided test on proportion**
- **Sample Size: ~ 600 total specimens**

Specimen Identification



Results

1:1 population

Concordance = 82% (95% CI of 78%, 85%)

κ statistic = 0.64 (95% CI of 0.58, 0.70)

	CTA -	CTA +
FISH -	**235**	**88**
FISH +	9	**197**
TOTAL	244	285

Concordance Study

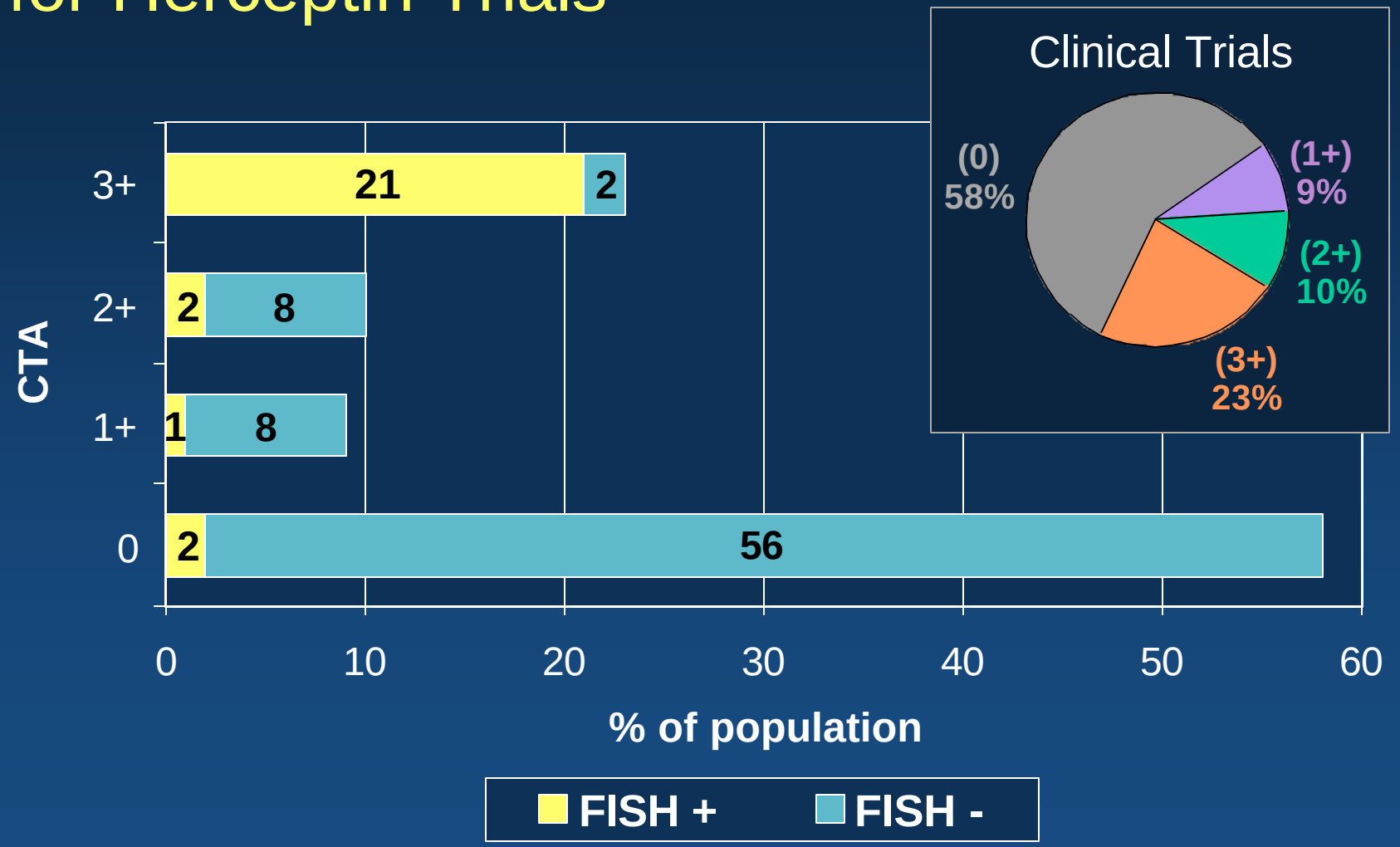
Results

1:1 population

		CTA 0	CTA 1+	CTA 2+	CTA 3+
FISH	-	207	28	**67**	21
	+	7	2	21	176
Amplification rate		**3%**	**7%**	**24%**	**89%**

Concordance Study

Extrapolation to the Population Screened for Herceptin Trials



Results

Extrapolated to Clinical Trials Population

Concordance = 88% (95% CI of 85%, 91%)

	CTA -	CTA +
FISH -	**342**	53
FISH +	12	**122**
TOTAL	354	175

PathVysion versus HercepTest

	1:1 concordance	Extrapolated concordance
PathVysion/CTA	**82%**	**88%**
HercepTest/CTA	**79%**	**83%**

Conclusions

- The concordance between PathVysion and the CTA in a 1:1 population is 82%
- This exceeded the pre-specified level of acceptability ($p < 0.0001$)
- The level of concordance between PathVysion and the CTA is consistent with HercepTest
- PathVysion will provide similar performance, compared to HercepTest, when used as a surrogate for the CTA to select patients for Herceptin therapy

Clinical Outcomes Analysis

Rationale

- Post-approval commitment to the FDA to explore other HER2 diagnostics in the context of Herceptin clinical trials
- Provide clinical outcomes data, in addition to concordance, to support FISH as an appropriate method to select patients for Herceptin therapy

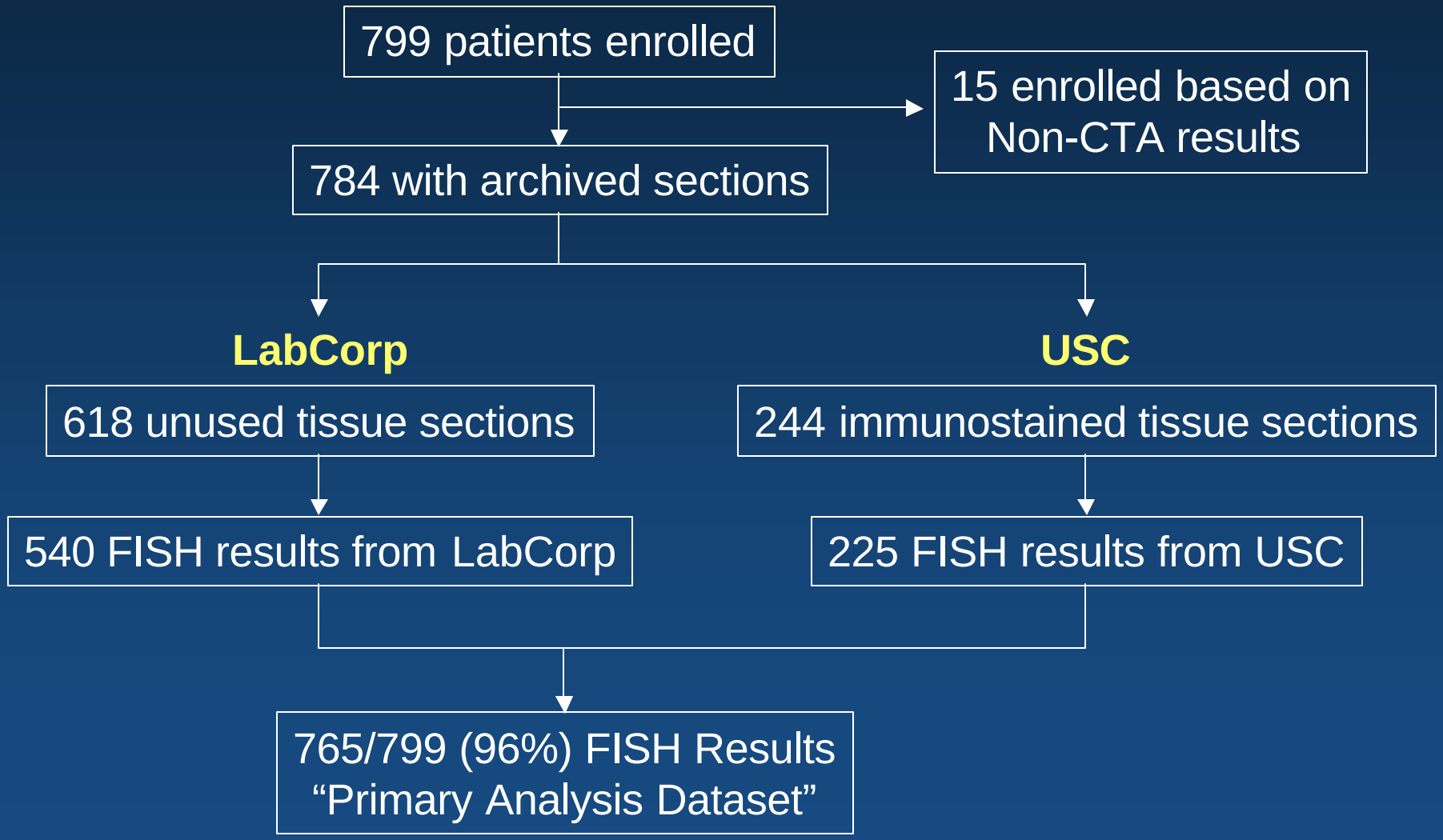
Objective

- Explore the relationship between FISH status (FISH+ versus FISH-) and Herceptin clinical benefit as assessed by a retrospective analysis of:
  - Response rate
  - Time to disease progression
  - Survival
- In 3 Herceptin clinical trials (n=799 patients)
  - Chemotherapy +/- Herceptin, 1st line MBC
  - Herceptin monotherapy, 2nd & 3rd line MBC
  - Herceptin monotherapy, 1st line MBC

Study Population

- The Herceptin pivotal trials represent the only large database available to correlate HER2 diagnostics with treatment outcome
  - Tissue database was not designed for subsequent validation of alternative diagnostic assays
  - Tumor blocks or tissue sections submitted: *only* tissue sections archived
  - Clinical outcomes data available only for the CTA 2+/3+ subset who enrolled into Herceptin trials

Specimen Identification



Study Design

Herceptin monotherapy, 2nd & 3rd line MBC

Eligible Patients (n = 222)

- Metastatic breast cancer
- HER2 overexpression (2+/3+)
- 1 or 2 prior CT for MBC anthracycline and taxane

Herceptin
4 mg/kg loading dose
2 mg/kg/wk maintenance

Primary Endpoint:	**Response Rate**
Secondary Endpoints:	**Time to Progression** **Survival**

Response Rate

Herceptin monotherapy, 2nd & 3rd line MBC

	n	Herceptin monotherapy
FISH +	33/163	**20%** (14.4, 27.2)
FISH -	0/46	**0%** (0.0, 7.7)

2+/3+ 15%

Study Design

Chemotherapy +/- Herceptin, 1st line MBC

Eligible Patients (n = 469)

- Metastatic breast cancer
- HER2 overexpression (2+/3+)
- No prior CT for MBC

Chemotherapy + Herceptin

Chemotherapy Alone

Primary Endpoint:	**Time to Progression**
Secondary Endpoints:	**Response Rate** **Survival**

Response Rate

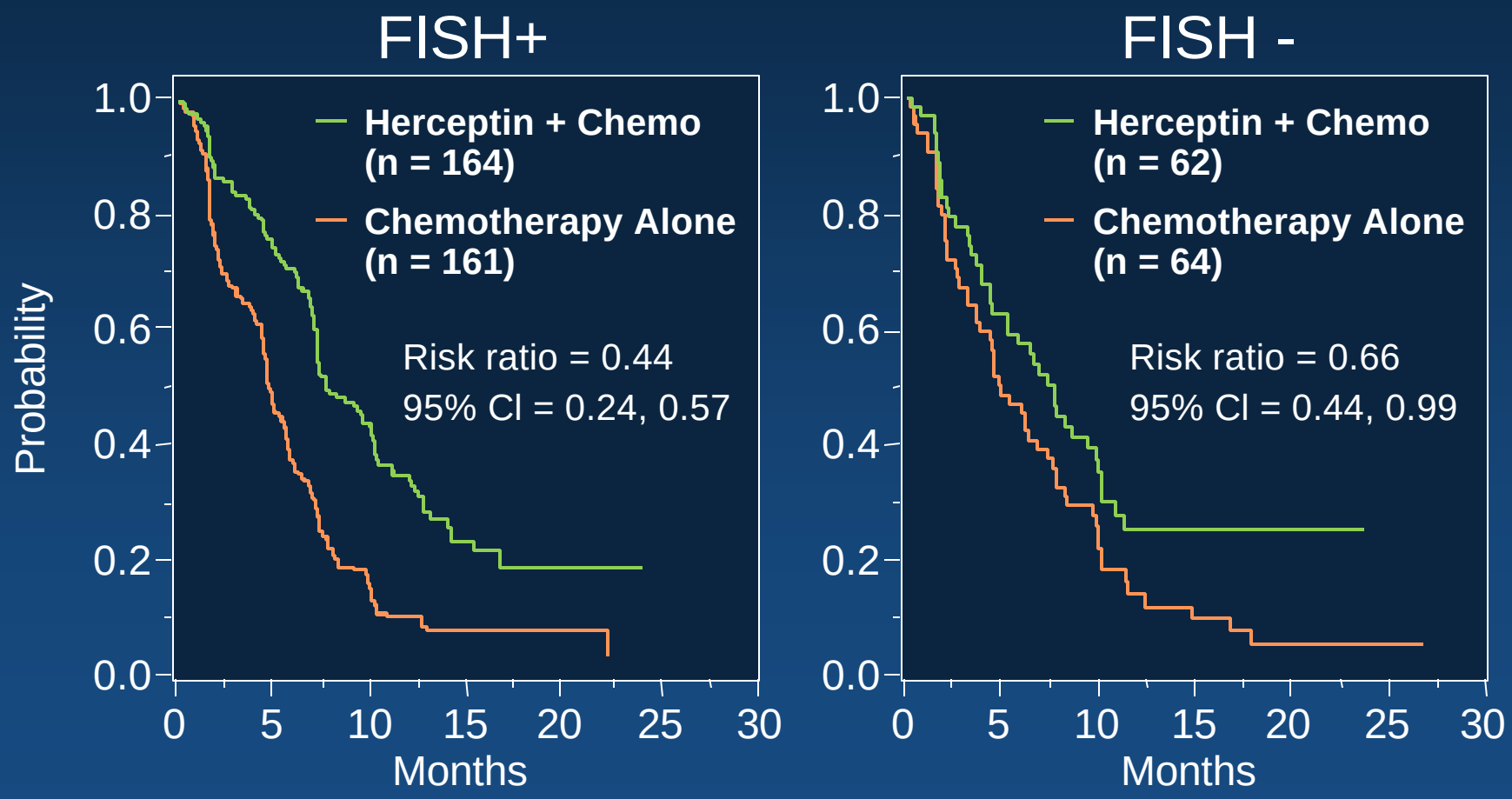
Chemotherapy +/- Herceptin, 1st line MBC

	Chemotherapy alone	Chemotherapy + Herceptin
FISH + n=325	**30%**	**54%**
	p < 0.0001	
FISH - n=126	**38%**	**40%**
	p = 0.7452	

2+/3+
32→50%

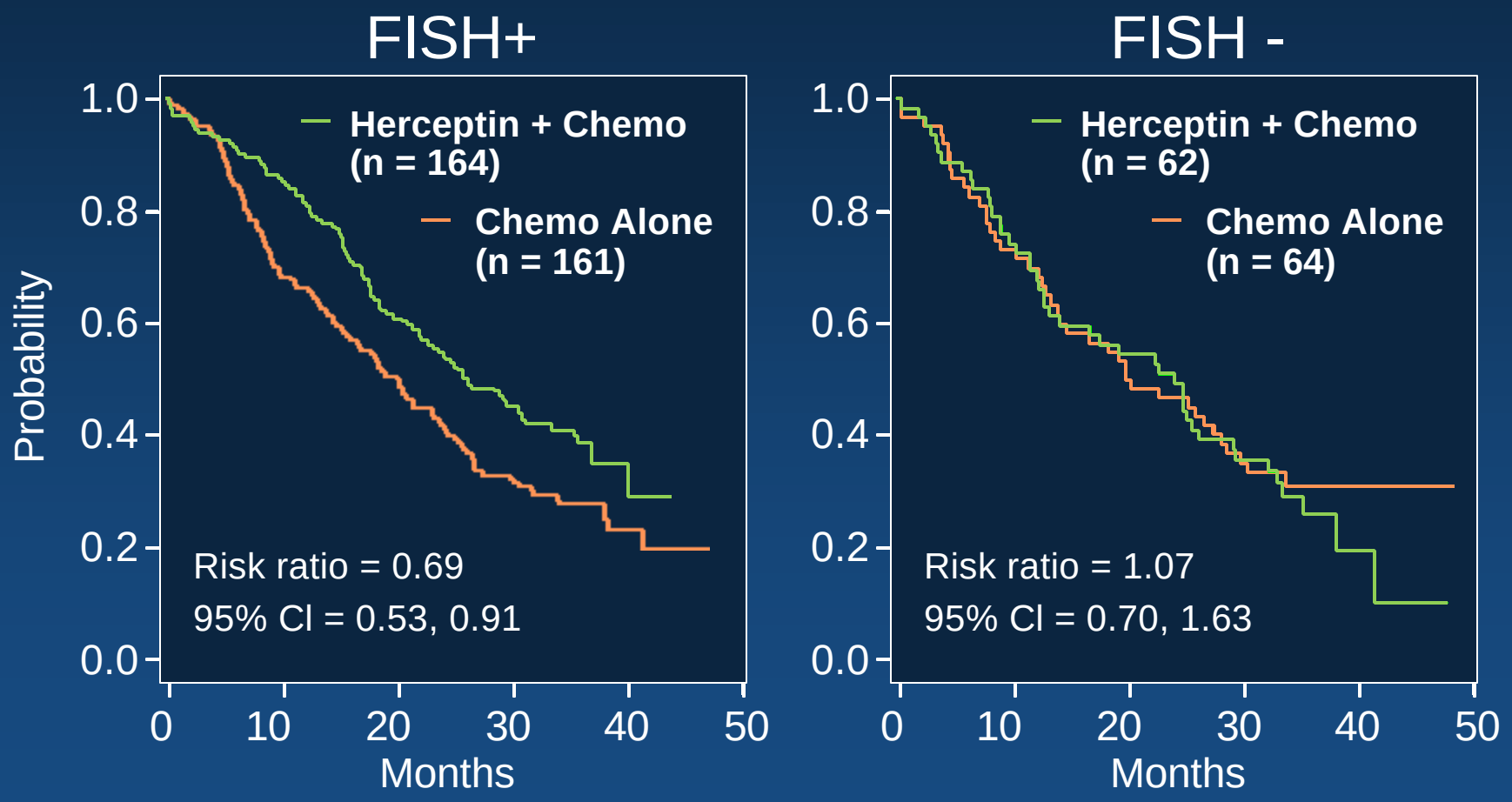
Time to Disease Progression

Chemotherapy +/- Herceptin, 1st line MBC



Survival

Chemotherapy +/- Herceptin, 1st line MBC



Summary

Within both pivotal trials FISH+ status appears to consistently identify a population which benefits from Herceptin therapy

	FISH (+)	FISH (-)
Monotherapy trial		
Response rate	20%	0%
Combination trial		
Response rate	30 → 54%	38 → 40%
Time to progression, risk ratio	0.44	0.66
Survival, risk ratio	0.69	1.07

Inter-laboratory Validation Assessment

- Objective
  - To ensure that assay methodology differences between the laboratories would not influence the interpretation of the clinical outcome results
- Methods
  - Previously immunostained tissue sections from 248 patients with known FISH results at LabCorp were sent to USC for repeat FISH testing in two stages
  - All patients with a FISH- result at LabCorp were retested at USC
  - Results obtained in 221/248 (89%)

Inter-laboratory Validation Assessment

- Results
  - Overall agreement 82%
  - LabCorp FISH+ agreement 98% (79/81)
  - LabCorp FISH- agreement 74% (103/140)
    - 84% of the 37 discordant results were CTA 3+
    - Indicative of underscoring at LabCorp
- Exploration of laboratory differences suggests
  - Different condition of the specimens
  - Different methodology for protease digestion step

Exploratory Secondary Analysis

- Re-analysis of the clinical data using USC results, when available
- No impact on the results of clinical outcomes analysis

	FISH (+)		FISH (-)	
	primary	secondary	primary	secondary
Monotherapy trial				
Response rate	20%	19%	0%	0%
Combination trial				
Response rate	30→54%	31→54%	38→40%	38→38%
Time to progression risk ratio	0.44	0.45	0.66	0.68
Survival risk ratio	0.69	0.70	1.07	1.13

Unanswered Questions

- Do FISH+ / IHC (0,1+, 2+) benefit to the same extent as FISH+ / IHC 3+?
- Do FISH- / IHC 3+ benefit to the same extent as FISH+ / IHC 3+?
- What can be concluded regarding these subsets from retrospective analyses of the Herceptin pivotal trials?
- Are prospective clinical trials feasible?

Clinical Outcomes Analysis

Do FISH+ / IHC (0,1+, 2+) benefit to the same extent as FISH+ / IHC 3+?

FISH	CTA 0	CTA 1+	CTA 2+	CTA 3+
-	56%	8%	8%	2%
+	**2%**	**1%**	**2%**	21%

Expected Distribution in Clinical Trials Population

Prospective Confirmatory Trials

- Metastatic Breast Cancer
  - Assumptions:
    - Comparison is 3+/FISH+ versus <3+/FISH+
    - Non-inferiority design

Required Number of Screened Patients	Required Sample Size
~30,000	3300

Summary

- The concordance analysis demonstrates that PathVysion will provide similar performance, compared to HercepTest, when used as a surrogate for the CTA to select patients for Herceptin therapy
- The clinical outcomes analysis provides additional data supporting FISH as an appropriate method to select patients for Herceptin therapy

Final Conclusion

The Herceptin package insert should be modified to include PathVysion as an appropriate method to aid in the selection of patients for Herceptin therapy